

RECENT NEWS ON TRICHINELLOSIS : ANOTHER OUTBREAK DUE TO HORSEMEAT CONSUMPTION IN FRANCE IN 1993

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Summary :

A new outbreak of trichinellosis occurred in France in December 1993 and involved around 550 patients. The authors report here how recent knowledge on *Trichinella* have been helpful to investigate this outbreak.

KEY WORDS : *Trichinella*, trichinellosis.

In December 1993, a new outbreak of trichinellosis in France proved once again that *Trichinella* was still a preoccupating public health problem. A total of 538 cases were infected in November 1993 in 9 foci : 18th district of Paris, Velizy (Yvelines), La Rochelle (Charente Maritime), Coulommiers (Seine et Marne) and five scattered foci in various parisian districts. All patients (except one) had eaten horse-meat during the first fortnight of November. Subsequent epidemiologic and veterinary inquiries have shown that all these foci were related to a single horse carcass imported from Canada. The responsibility of horse meat was proven by case-control studies (Ancelle, 1994), similar to those carried out in 1985 (Ancelle *et al.*, 1988). Unfortunately, the indisputable evidence of horse-meat responsibility, i.e. the discovery of larvae in meat, was lacking on account of the long incubation of symptoms (3 weeks). When a trichinellosis outbreak occurs veterinary authorities usually examine thousands of samples taken from carcasses still present in butchereries ; but an extensive search of meat bought 2 to 3 weeks before and possibly deep-frozen by consumers would usefully complete these examinations.

The experience acquired in 1985 allowed us to investigate these outbreaks very quickly and to advise early specific treatments. Firstly, we had shown in 1985 that the investigation of a trichinellosis epidemic focus could be done by interrogating private labora-

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Résumé :

Une nouvelle épidémie de trichinellose a éclaté en France en décembre 1993 et a touché environ 550 malades. Les auteurs évoquent ici les connaissances récentes sur le parasite qui ont été utiles dans la prise en charge de cette épidémie.

MOTS CLES : *Trichinella*, trichinellose.

tories located in the vicinity of the first cases (Ancelle, 1991). A higher frequency of hypereosinophilia on white blood cell counts is an important biological event to suspect a focus. Secondly, since 1975 and including this recent episode, nine outbreaks from horse origin have been described in France and Italy (table I) and no time was lost to convince professionals and sanitary authorities of horse-meat responsibility. Moreover, experimental works have shown that horses were susceptible to infection of different *Trichinella* isolates and could harbour important numbers of larvae in their muscles (Soulé *et al.*, 1989, 1993a).

The knowledge of the genus *Trichinella* has greatly increased since the works of Pozio and co-workers who described, after analysis of 27 enzymes of 152 isolates from 5 continents, eight types of *Trichinella* (table II). Five of these patterns represent the five species described so far : *Trichinella spiralis* (T1), *T. nativa* (T2), *T. pseudospiralis* (T4), *T. nelsoni* (T7) and *T. britovi* (T3) (Pozio *et al.*, 1992; La Rosa *et al.*, 1992). One drawback of this technique is the number of enzymes to analyse to distinguish the different species. DNA analysis has also been proposed to compare different isolates by means of restriction fragment length polymorphism (Klassen *et al.*, 1986; Dick *et al.*, 1990; Zarlenga *et al.*, 1991) or polymerase chain reaction (Dupouy-Camet *et al.*, 1991; Dick *et al.*, 1992; Soulé *et al.*, 1993b). However, these techniques, useful to differentiate some isolates, could not easily identify the different species. Recent advances in genetic research have included the development of an adaptation of the polymerase chain reaction known as the random amplified polymorphic DNA polymerase chain reaction (RAPD). The technique is fast, easy to perform, requires a small amount of DNA

and no prior sequence information and is based on the use of a single synthetic 10-mer oligodeoxynucleotides. The primer is short, increasing the probability that two priming sites occur in the genome close to each other in inverted orientation. Competition between many initial primers extension products and a low annealing temperature results in the synthesis of few intense DNA bands after amplification. We and others have shown its usefulness to type *Trichinella* isolates (Bandi *et al.*, 1993a; Dupouy-Camet *et al.*, 1993). Patterns obtained for *T. nelsoni*, *T. spiralis* and *T. pseudospiralis* were quite different from each other and from our analysis, T5 was not very different from some isolates of *T. britovi* and *T. nativa*. The possibility to type single larva by RAPD gave the opportunity to prove that the same host could be infected by two sympatric species (Bandi *et al.*, 1993b).

In the last outbreak in France, the parasite burden in the two muscular biopsies carried out, was too low (1-2 larvae/120mg) to obtain the isolate on mice (however, the isolate was obtained from a biopsy carried out in a cat living in a family of patients). With DNA of a single larva and by conventional PCR and RAPD, E. Pozio (Istituto Superiore di Sanita, Rome, Italy) typed this isolate as *T. spiralis*.

The precise identification of isolates responsible for human outbreaks have also shown that clinical or biological characteristics were linked to the consid-

ered species : e.g. Pozio *et al.* (1993) reported that, with similar infective doses, *T. britovi* induced a milder symptomatology in humans and gave lower antibody titers than *T. spiralis*. These differences of infectivity could be related to differences of antigenicity (Dupouy-Camet *et al.*, 1988; Bolas-Fernandez & Wakelin, 1989). In the recent french outbreak, the symptomatology was moderate, the apparition of antibodies was delayed (up to 2 weeks after fever, myalgias and facial oedema). Considering the north-american origin of the horse, a T5 isolate (as in August 1985) was suspected (Snyder *et al.*, 1993) but this hypothesis was not confirmed by the subsequent analysis. The mild symptomatology and the delay of seroconversion were then probably related to a small infective dose of parasites. An accurate comparison of clinical and biological signs in different outbreaks requires the use of a uniform record as proposed by the International Commission on Trichinellosis.

In 1985, late myalgias were less frequent in individuals treated with tiabendazole (Bourée, 1991). Therefore, in the last outbreaks, physicians were advised to treat their patients by tiabendazole. Unfortunately, the obtention of this non commercialized drug had been difficult for some non hospitalized patients. Moreover, several severe cutaneous reactions and the bad general tolerance was preoccupating and led some physicians to use albendazole; the efficacy of this drug could be higher (Fourestié *et al.*,

Date	Place	Number of cases	Horse origin	Type of isolate	Reference
1975	Bagnolo in Piano, Italy	89	Yugoslavia	<i>T. britovi</i>	Mantovani <i>et al.</i> , 1980
1975	Chatenay-Malabry, France	125	East Europa	-	Bourée <i>et al.</i> , 1979
1984	Varese, Italy	13	Yugoslavia	-	Parravicini <i>et al.</i> , 1986
1985	Paris & Melun, France	431	USA	T5	Ancelle <i>et al.</i> , 1988
1985	Paris & 10 foci, France	642	East Europa (Poland ?)	<i>T. spiralis</i>	Ancelle <i>et al.</i> , 1988
1986	Salsomaggiore, Italy	> 300	East Europa	<i>T. britovi</i>	Pozio <i>et al.</i> , 1988
1991	Barletta, Italy	> 500	East Europa	<i>T. spiralis</i>	Pozio, pers. comm., 1993
1991	Clermont-Ferrand, France	21	USA	-	Beytout <i>et al.</i> , 1991
1993	Paris, La Rochelle, & 2 foci, France	538	Canada	<i>T. spiralis</i>	Ancelle <i>et al.</i> , 1994

Table I. - Outbreaks of horse-meat related trichinellosis in Europa, 1975-1994.

More than 2600 individuals were involved in trichinellosis outbreaks. Five deaths were reported during the french outbreaks of 1985.

type	T1	T2	T3	T4	T5	T6	T7	T8
species	<i>T. spiralis</i>	<i>T. nativa</i>	<i>T. britovi</i>	<i>T. pseudospiralis</i>	(3)	(4)	<i>T. nelsoni</i>	(3)
geographical repartition	cosmopolitan	holoarctic	Europa, Asia	cosmopolitan	North America	North America	Africa	South Africa
climate	varying	cold	temperate	temperate	temperate	cold	tropical	sub-tropical
hosts	pig, dog, cat, fox	wolf, bear	fox, rat, pig	marsupials, birds	bear, racoon	bear, wolf	hyena, lion	hyena, lion
Wistar rat susceptibility	+++	0	+/-	++(1)	+/-	0	+/-	+/-
freezing resistance	0	+++	+	0	0	++	0	0
found in man	+	+	+	+(2)	+	?	+	?

Table II. The 8 types of *Trichinella* determined by isoenzyme analysis (data from Pozio, 1992).

1. *T. pseudospiralis* is non encapsulated. 2. One human *T. pseudospiralis* infection was reported in New-Zeland (Andrews *et al.*, 1993). 3. T5 and T8 are similar to *T. britovi*. 4. T6 is similar to *T. nativa*.

1988). The use of corticosteroids should be encouraged since Fourestié *et al.* reported in 1993 that an important number of circulating eosinophils could correlate with neurological and cardiac complications and that corticosteroids could prevent this event. The number of neurological and cardiac complications were very low in this recent episode and no death were reported. Moreover, in our opinion, hospitalisation should be reserved to severe cases where patients require a constant surveillance. Mild forms can be monitored by general practitioners.

If antibodies and circulating antigens evolution are well known (Van Knapen *et al.*, 1982; Ljungström *et al.*, 1988; Ivanoska *et al.*, 1989), effects of *Trichinella* on the human cellular immune system are speculative. Recently, a significant lymphocytopenia involving T4 and T8 cells was reported in humans during the first week of fever when new-born larvae migrate (Dupouy-Camet *et al.*, 1994). Experimental studies in mice infected by *T. spiralis* showed a significant decrease of CD4 lymphocytes and of CD4/CD8 ratio, two weeks after infection (unpublished data). This lymphocytopenia could result from a destruction of lymphocytes by factors from parasite or host origin (lymphocytotoxin described by Faubert & Tanner, 1975) and/or a sequestration of lymphocytes in perilarval granulomas (Neiffer *et al.*, 1991). This lymphocytopenia could facilitate the survival of the migrating larvae as previously shown in mice treated by an anti-lymphocyte serum (Karmanska & Michalska, 1981). Attempts to assay cytokines in sera from patients were disappointing (Gari-Toussaint, pers. comm., 1993) and, though not proven, important

amounts of TNF could be found in serum. Experimentally, *Trichinella* induces a sequential secretion of Th2 derived cytokines (IL3, IL4, IL5 etc) activated by adults antigens and of Th1 derived cytokines (TNF, IL2 etc) activated by newborn larvae (Zhu & Bell, 1989; Pozio & Brushi, 1994).

The main question about these horse-meat related outbreaks concerns the natural mode of horses infection. Could horses be infected by the ingestion of faeces containing larvae or adults expelled by refractory or immune hosts? Could these larvae be ingested and protected in ground insects (Campbell, 1983)? The low number of larvae which could be transmitted in this way appears too small to infect a horse: in experimental infections 5000 larvae given to a horse produced a few larvae in 10 grammes of muscles (Soulé *et al.*, 1989). A probable hypothesis is the accidental ingestion of a contaminated rodents or meat, ground up with hay or food. An american horse breeder reported to us the finding of minced rabbit or sheep carcasses in hay cut up as food (I. Einstein, pers. com. 1994). Moreover, since 1985 when the search for *Trichinella* in horse-meat became compulsory, not a single carcass has been found positive though millions have been examined and in spite of this control, 4 outbreaks were reported in the european community. There are anecdotal findings of isolated larvae after artificial digestion horse-meat pieces but since 1897 (Thornbury, 1897), *Trichinella* larvae have never been observed microscopically in horse-meat. The constantly negative controls could lead to discouragement and disaffection of technicians in charge of these techniques, the-

before they should be assessed by regular training. In the recent outbreak, considering the theoretical prolificity of *T. spiralis* females, the number of larvae found in human biopsies and the mean consumption of meat (150 grammes), the number of larvae in horse-meat could have been as small as 2 larvae per gramme. The limit of sensitivity of trichinostomy is considered to be 3 larvae per gramme (Touratier, 1991). The amount of meat required by regulations for pooled digestion of one hundred samples, is one gramme; finding 2 larvae in these conditions seems unlikely. Digestion of 5 to 10 grammes of meat per carcasses should be a real improvement and this was the recent conclusion of a working group of the European Union. Alternative methods of control have been experimentally analyzed. If PCR was an interesting model in a mouse model, a large use for control seems unrealistic at present (Dupouy-Camet *et al.*, 1991). Results obtained with circulating antigens in horses were delicate to interpretate; specific antibodies assayed by indirect immunofluorescence or ELISA disappeared about 6 months after infection though larvae were still present in muscles (Soulé *et al.*, 1989, 1993). The cost of such procedures should be compared to the social cost of the disease : 7 to 11 millions francs for the two french outbreaks of 1985 (Ancelle *et al.*, 1990).

Sufficient cooking of meat is an efficient prophylactic method but french consumers do prefer their horse-meat raw or rare. Therefore, consumers could be advised to deep-freeze their meat for 10 to 15 days (Touratier, 1991). The important number of patients involved in horse-meat related outbreaks have somewhat hidden other sources of infection ; however, autochthonous wild boars are still responsible for family outbreaks in the south of France (117 patients in 9 outbreaks since 1977).

Though being a public health threat, *Trichinella* is a fascinating model. If the intestinal phase has been extensively studied, physiopathological studies of the muscular phase are more recent. A few hours after penetration of the new born larvae, the muscular fiber is disorganized : myofilaments have disappeared, nuclei are modified and the outer membrane increases by 20-30 folds (Jasmers *et al.*, 1991). The precise mechanism is yet unclear but proteins from parasitic origin have been found in the muscular fiber nuclei and a *Trichinella* MyoD homologue could interfere with the regulation of the endogenous muscle genes (Despommiers, 1990; Pozio & Bruschi, 1994). The parasite is in an intracytoplasmic position and takes control of the whole metabolism of the host-cell. We agree with Despommier's statement qualifying *Trichinella* "a worm that would be virus".

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